FLAVOR COMPONENT EVALUATION
ANNUAL REPORT

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TABLE OF CONTENTS

		Page No.
I.	OBJECTIVES	1
II.	SUMMARY	2
III.	TOBACCO FLAVOR	4
	A. VOLATILE NEUTRAL FRACTION OF BRIGHT AND BURLEY TOBACCOS	4
	B. NONVOLATILE FRACTIONS OF TOBACCO	6
	C. SMOKE FLAVOR RESPONSE - BURLEY TOBACCO	10
IV.	PURGE & TRAP/GAS CHROMATOGRAPHIC ANALYSIS	16
	A. PLAYERS AND RAFFLES	16
	B. MARLBORO 85	20
	C. COUMARIN IN CIGARETTES	21
	D. MISCELLANEOUS APPLICATIONS	21
V.	NICOTINE TRANSFER TO SMOKE	25
VI.	CASINGS AND FLAVORS	30
	A. VANILLA FLAVOR REPLACEMENT	30
	B. TONKA BEANS	30
	C. COCOA SHELLS	32
VII.	PLANS	34
VIII.	ACKNOWLEDGMENTS	35
IX.	REFERENCES	36

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I. OBJECTIVES - FLAVOR COMPONENT EVALUATION (2306)

(1) Identify important flavor constituents of tobacco and smoke and determine their role in smoke flavor.

(2) Study the transfer of flavor compounds from the cigarette rod to smoke.

(3) Separate and identify components in selected flavors presently used in Manufacturing to assist Flavor Development Group in the formulation of substitute flavors.

(4) Assist R&D groups and Manufacturing with flavor problems.

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II. SUMMARY

The work during the past several years on the volatile neutral fractions from Bright and Burley tobaccos is reviewed. Over 200 compounds were tentatively identified. These compounds were found in both Bright and Burley tobaccos, and only the ratios of these compounds were different.

The nonvolatile compounds of tobacco are very complex. The compounds were not sufficiently resolved by HPLC separation to permit identification even with an initial preseparation by column chromatography.

The smoke flavor response of Burley tobacco was investigated using an "electric cigarette." Since a large amount of ammonia was detected from the "electric cigarette," the application of ammonia to Bright tobacco was reexamined. The pH of smoke from various tobaccos was determined as a possible explanation of the choking sensation of Burley smoke. Work was initiated on evaluation of the subjective response to ultrasonically generated aerosols to determine the effects of free nicotine and some of its salts.

The purge and trap GC analysis has shown the transfer of volatile compounds from the packaging material to Players and Raffles cigarettes. The major material transferring to the cigarettes are the solvent residues from the printing inks. This technique has also been used to analyze coumarin in competitor's cigarettes.

The problem of the high nicotine transfer to smoke of PM cigarettes in Brazil is discussed. Work was carried out to determine the reason for this more efficient nicotine transfer to smoke. Literature is cited where the lower the potassium content in the tobacco the higher

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the nicotine in smoke.

The volatile components of vanilla and tonka beans were isolated and identified to aid in a formulation of flavors for cigarettes. An investigation was also carried out on the volatile compounds of cocoa shells.

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III. TOBACCO FLAVOR

A. Volatile Neutral Fraction of Bright and Burley Tobaccos

Mr. Hale has summarized the work he has carried out during the past several years in a Special Report #83-012 titled, "Identification of the Components in the Volatile Neutral Fractions of Bright and Burley Tobacco." In this work Bright and Burley tobaccos were extracted with methylene chloride. The extracts were separated into acid, base and neutral fractions. The neutral fractions were steam distilled and the distillate separated by silicic acid column chromatography into 12-15 fractions. These fractions were analyzed directly by capillary column GC/MS. For identification of material in small quantities, fractions were initially separated on a packed column and peaks or areas where these minor components eluted were trapped and reanalyzed by capillary column GC/MS.

Over 200 compounds were tentatively identified by GC/MS and are listed in the report. It was observed that the same components are present in both Burley and Bright tobaccos and only the ratios of these components are different. It is felt that many of the components may make little or no contribution to tobacco odor but may be important for smoke flavor response. One of our goals is to seek better methods for screening and evaluating tobacco components for their odor and smoke flavor response.

A unique technique for selecting the odorous components was developed by Hale. Each sample was run by gas chromatography using a 6 meter packed column equipped with an exit splitter for odor evaluation by members of the group. Each peak eluting from the chromatograph was evaluated by odor and the comments recorded on the scan.

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Selected fractions were rerun on the chromatograph and those peaks having high odor intensities were trapped in capillary tubes with dry ice. These tubes were sealed in small test tubes. A small portion of the original fraction was also injected into a capillary tube and sealed in a test tube. Each tube was evaluated for odor and selected tubes combined to reproduce the odor of the original fraction. The tubes are added or removed until the odor matches that of the original fraction. Most of the time this method will eliminate over half of the components that were trapped. The tubes containing the selected peaks were then run by GC/MS for identification. This method reduced interferences from so many components present in the original fraction. This is also a good method of concentrating a selected component for GC/MS.

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B. Nonvolatile Fractions of Tobacco[1]

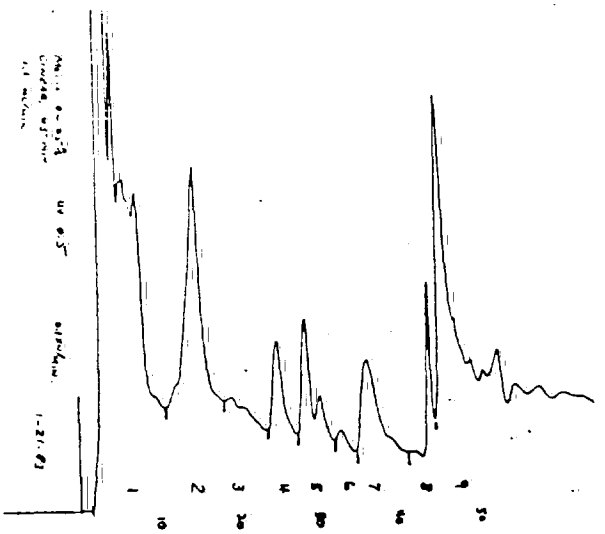
1. Bright Tobacco

Last year's annual report described the work on the nonvolatile methylene chloride extract of Zimbabwe flue cured tobacco. The methylene chloride extract was separated by silicic acid column chromatography and followed by two separations on reverse phase HPLC. Only fraction 5-3-5 was found to impart a fuller smoke. Unfortunately this fraction was the smallest one among the six. Normal phase HPLC separation using silica gel column was carried out and the two larger peaks collected turned out to be phthalates of long chain fatty acids.

2. Burley Tobacco

The water extract of Burley tobacco which has been previously extracted with methylene chloride was freeze dried. The freeze dried powder was re-dissolved in 50% methanol and separated by reverse phase HPLC (Figure 1). Nine fractions were collected. Attempts to identify the compounds present in each fraction was not successful since each fraction was a mixture.

FIGURE 1
HPLC SEPARATION OF WATER SOLUBLE FRACTION
FROM BURLEY TOBACCO



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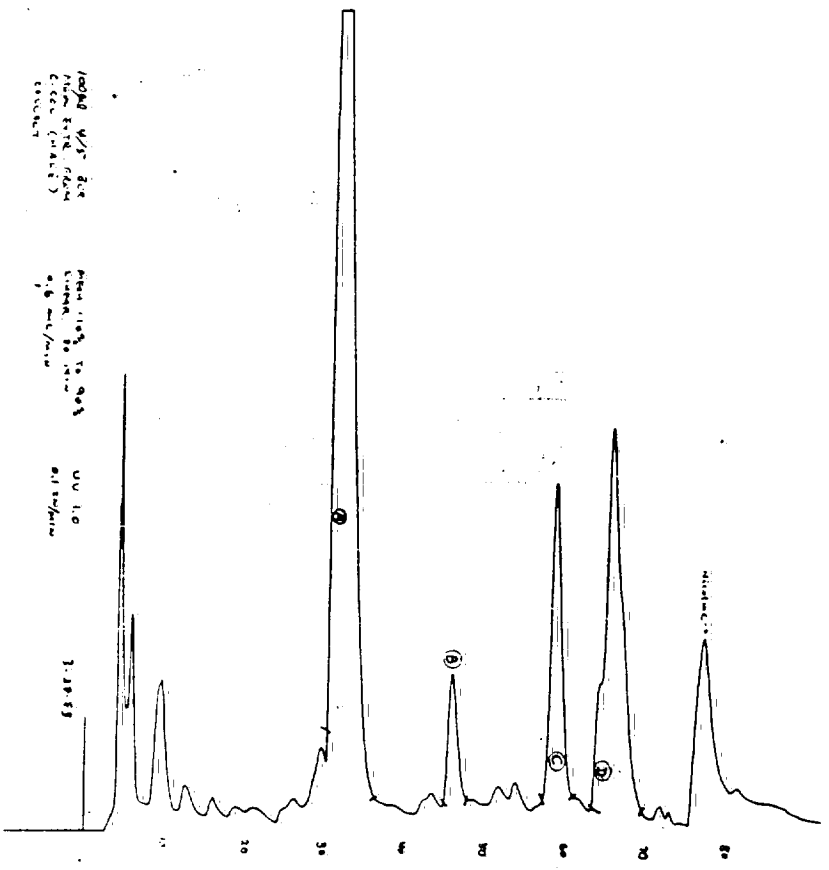
Methanol extract of Burley tobacco was also obtained. It was initially separated into twelve fractions by a carbon column. Each fraction was analyzed by reverse phase HPLC. Fractions 11 and 12 were found to contain insignificant amounts of material. The chromatograms of all fractions indicated significant overlapping of peaks. Fractions 4 and 5 were combined and separated by reverse phase HPLC (Figure 2).

FIGURE 2

HPLC SEPARATION OF A COMBINED FRACTION OF FRACTIONS 4 AND 5



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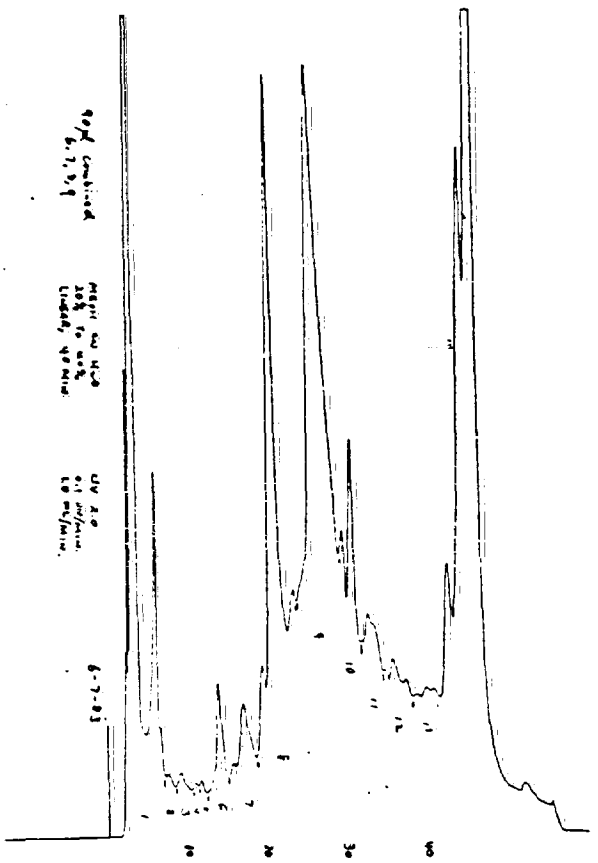
Four major peaks were collected. Each peak was again purified by normal phase HPLC. Peaks A and D were collected and submitted to the Analytical Division for identification. Peaks B and C resolved into a group of small peaks. It was decided that identification of major peaks would be carried out first. Fractions 6, 7, 8 and 9 were combined and separated by reverse phase HPLC (Figure 3). The fractions resolved are being collected.

FIGURE 3

HPLC SEPARATION OF A COMBINED FRACTION OF FRACTIONS 6, 7, 8, AND 9



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Progress on the identification of the nonvolatile tobacco constituents has been slow. The water soluble fraction of tobacco appears to be very complex. This fact was reported in our last annual report and reported recently by Kroustalis[2] at his Annual Review. The capillary GC scans of the acid hydrolyses of the water soluble fraction is very complex when compared with our HPLC separation of the water soluble material. Therefore, it is not surprising that the material collected from HPLC separation have been reported to be mixtures. Our program on the water soluble fraction is being reevaluated.

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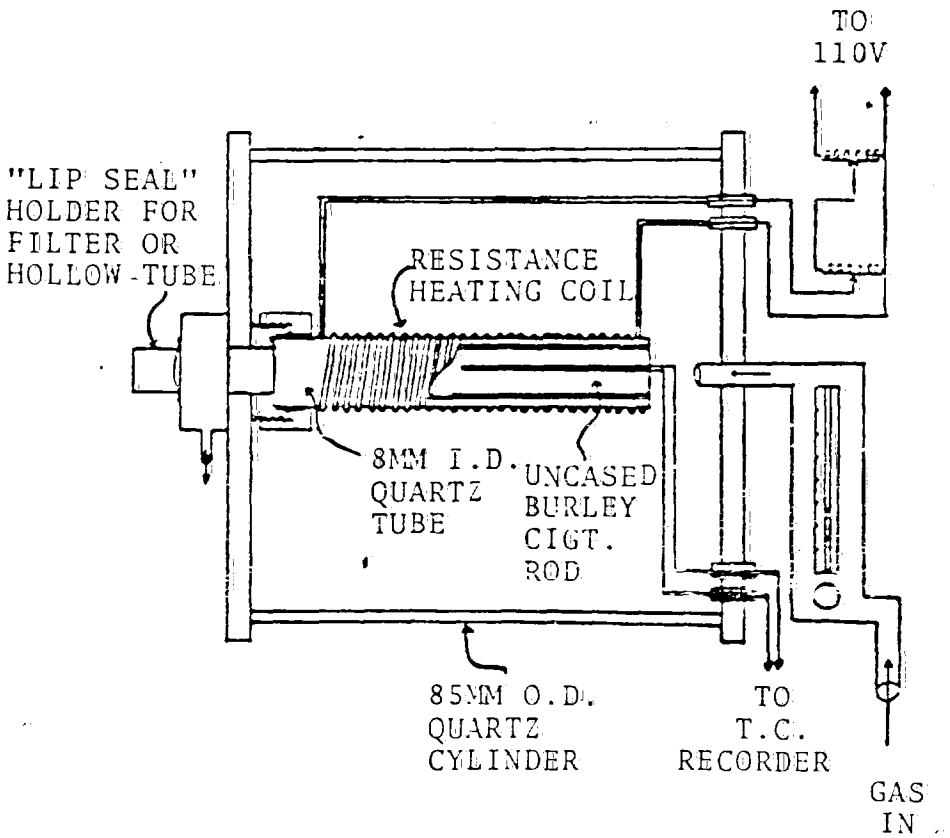
C. Smoke Flavor Response - Burley Tobacco[3]

A major goal for the Flavor Component Evaluation Group is to determine the important flavor components of tobacco and smoke. The recent emphasis on Burley tobacco has initiated a novel approach toward understanding the relationship between smoke flavor response and certain feeling factors associated with subjective response.

The electric cigarette as shown in Figure 4 is an apparatus to heat filler and to subjectively evaluate the eluted material.

FIGURE 4

ELECTRIC CIGARETTE



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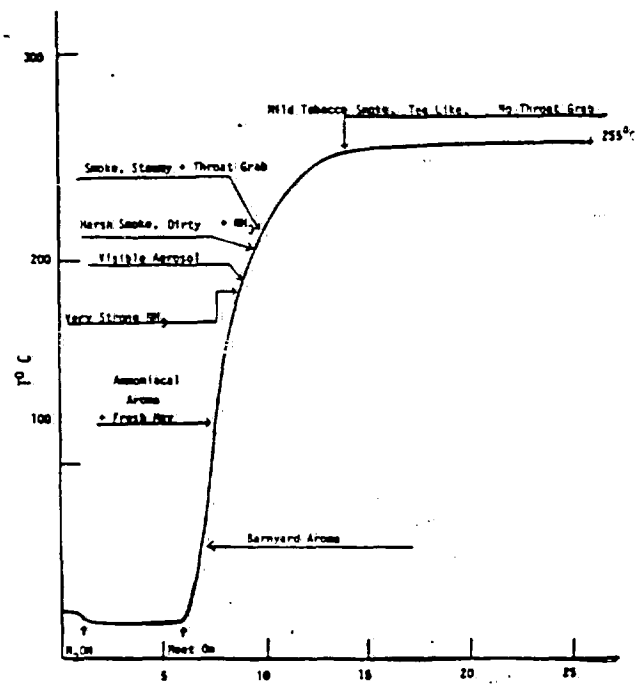
In this device, filler can be electrically heated at various rates in a flow of an inert gas. Basically this unit is a pyrolysis apparatus and instead of attaching it to an instrument, the effluent is sniffed by the nose or inhaled by the mouth for subjective evaluation. Burley tobacco was heated in the electric cigarette. Subjective responses detected during the heating of the Burley tobacco are labeled on the thermogram shown in Figure 5. This figure shows that the subjective responses of Burley smoke can be separated into various temperature intervals. The large amount of ammonia eluting from the heated tobacco was visually demonstrated by placing an open bottle of HCl at the exit port of the electric cigarette and observing the formation of a white cloud.

FIGURE 5

SUBJECTIVE RESPONSE ON HEATING BURLEY FILLER



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The electric cigarette was also used to volatilize the methylene chloride and water extracts of Burley tobacco. The harsh impact and most of Burley hay-like components were detected in the methylene chloride extract.

Because of the large amount of ammonia eluted from Burley tobacco on heating, and the association of this ammonia with certain Burley tobacco subjective characteristics, experiments were carried out on the treatment of Bright tobacco with ammonia. Keel[4] and Hale[5] had previously applied ammonia to Bright tobacco and found it to impart some of the choking sensation of Burley tobacco. Bright filler and all-Bright nonfiltered cigarette samples were placed in a cylinder and evacuated for various lengths of time. The moisture losses were determined, after which ammonia gas was bled into the evacuated cylinder. During the addition of ammonia a temperature rise in the tobacco was observed. The dryer the tobacco the higher the temperature rise. The ammonia pressure was maintained until the tobacco returned to room temperature. The amount of ammonia was measured by the weight gain. On venting the ammonia to atmospheric pressure and resealing, the cylinder containing the treated sample was heated at 110°C for various lengths of time. Ammonia treated samples were smoked for subjective evaluations. The choking sensation and throat harshness of Burley smoke were present but some of the aromatic qualities of Burley smoke were not present. Details of the work are reported in the Special Report #83-078 as one of the talks presented at the Burley Flavor Seminar by Lanzillotti.

Experiments on treating Bright tobacco with ammonia clearly indicated that the choking sensation and harshness can be enhanced.

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These same responses can be introduced by injecting ammonium hydroxide into the front of a cigarette filter prior to smoking. The Burley-like choking sensation produced by the addition of ammonia to Bright tobacco is consistent with the observation that the ammonia content of Burley tobacco is higher than Bright tobacco. The addition of ammonia to Bright tobacco increases the pH of water through which ammonia treated tobacco smoke has been bubbled. The pH values associated with the unfiltered smoke from cigarettes made from various tobaccos or treated tobaccos are shown in Table 1.

TABLE 1

UNFILTERED MAINSTREAM SMOKE pH

TOBACCO	WHOLE SMOKE	GAS PHASE	TPM
Oriental	5.0	4.8	5.8
Bright	5.6	4.8	5.9
Burley	6.0	4.5	7.7
Bright + NH_3	6.4	5.5	7.9
Bright + CaO	6.1	4.6	8.1

Several questions arose at this time:

(1) Is an increase in pH of the smoke by the application of ammonia to Bright tobacco responsible for the choking sensation and increased harshness?

(2) Is the buffer capacity of the fluid in the mouth sufficiently high that on smoking the pH does not change?

A simple test was carried out to determine the effect of smoking on the pH of the fluids on the tongue. The pH of the tongue was

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determined with pH indicator paper before and after smoking various cigarettes. These results are shown in Table 2.

TABLE 2

pH OF THE TONGUE
BEFORE AND AFTER SMOKING

	pH
Before Smoking	6-7
Oriental	6
Bright	6-7
Burley	8
Bright + NH_3	8

These results show that the change in pH of smoke changes the pH of the fluids on the tongue and therefore the subjective response can change.

Sidestream smoke has a high pH as shown in Table 3. Therefore, a device to inhale sidestream smoke was constructed. Inhaling sidestream Marlboro smoke did not choke - No Explanation!!

TABLE 3

UNFILTERED SIDESTREAM SMOKE pH

TOBACCO	WHOLE SMOKE	GAS PHASE	TPM
Oriental	7.7	8.0	7.8
Bright	8.2	8.1	8.1
Burley	8.9	8.3	8.5
Bright + NH_3	9.2	9.2	8.2
Bright + CaO	7.5	6.8	8.8

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A number of years ago an experiment was done to subjectively evaluate a nicotine tartrate aerosol generated by an ultrasonic transducer. This aerosol when inhaled imparted some of the choking sensation and the harshness in the back of the throat and in the mouth. To repeat this experiment and to subjectively evaluate nicotine aerosols at various pH's, an ultrasonic aerosol generator designed for administration of pulmonary medicine was purchased. The first test was to inhale distilled water. The majority of the people could not inhale the distilled water aerosol. A choking sensation was evoked and no harsh sensation. Discussion of this observation with various people revealed that to inhale the aerosol, a normal saline solution is necessary. The aerosol must have an ion content similar to natural body fluid (isotonic) in order to be inhaled. Plans are to inhale nicotine aerosol of various pH's to determine its subjective role in cigarette smoke.

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IV. PURGE AND TRAP/GAS CHROMATOGRAPHIC ANALYSIS (P&T/GC)[6]

The methodology for the P&T/GC analysis of the volatiles from tobacco filler and for other materials was described in the last annual report. During the past year the instrumentation was modified to separate the collected volatile components using two capillary columns. The incorporation of the second capillary column allows the sample to be separated simultaneously on polar and nonpolar columns.

A. Players and Raffles

Initial smoking of Players and Raffles cigarettes prior to test market indicated an off-flavor when compared with unpackaged cigarettes. P&T/GC analysis of the packaged cigarettes showed a large amount of very volatile components in the early portion of the gas chromatogram. These early peaks were much lower in the unpackaged cigarettes as shown in Figure 6. An extensive amount of work was carried out to determine the source of these early volatile components observed in the packaged cigarettes. This work has been reported by Allen Palmer in a Special Report #83-118, titled "The Role of Package Volatiles in Cigarettes Flavor: Players/Raffles Cigarettes."

Players 100 cigarettes were packed in both B&H Deluxe Ultra Lights and Players boxes. The two cigarettes were evaluated subjectively by the PED. The results from this showed no significant subjective difference between the cigarettes packaged in the two different boxes. P&T/GC analysis of these showed differences as shown in Figure 7.

One gram samples of Players box which had cigarettes, Raffles box which had no contact with cigarettes and an unprinted Tako board were analyzed by the P&T/GC analysis. These gas chromatograms are shown in Figure 8. The GC scan of the Players box which contained

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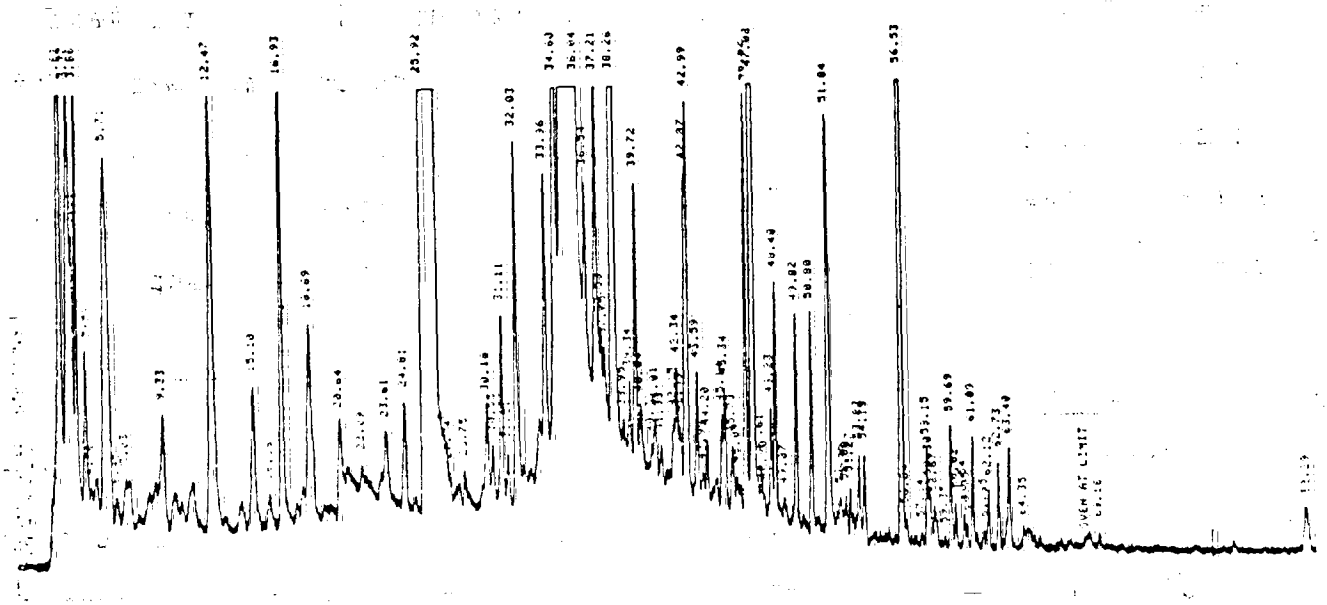
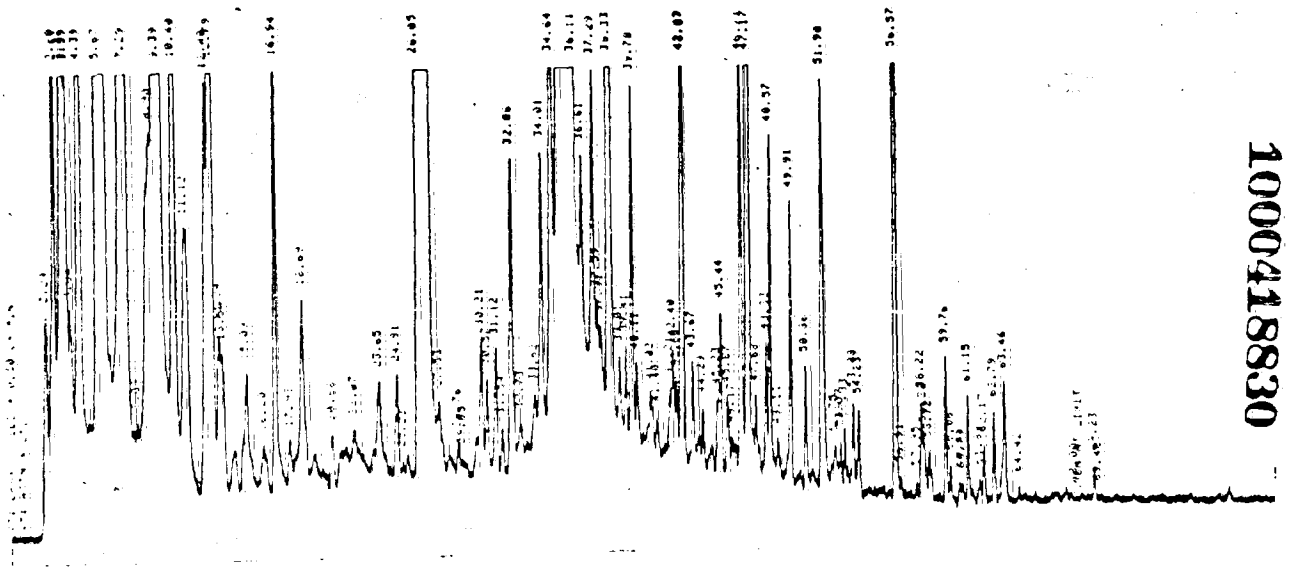
FIGURE 6

GAS CHROMATOGRAMS OF UNPACKAGED & PACKAGED PLAYERS CIGARETTES



UNPACKAGED



PACKAGED

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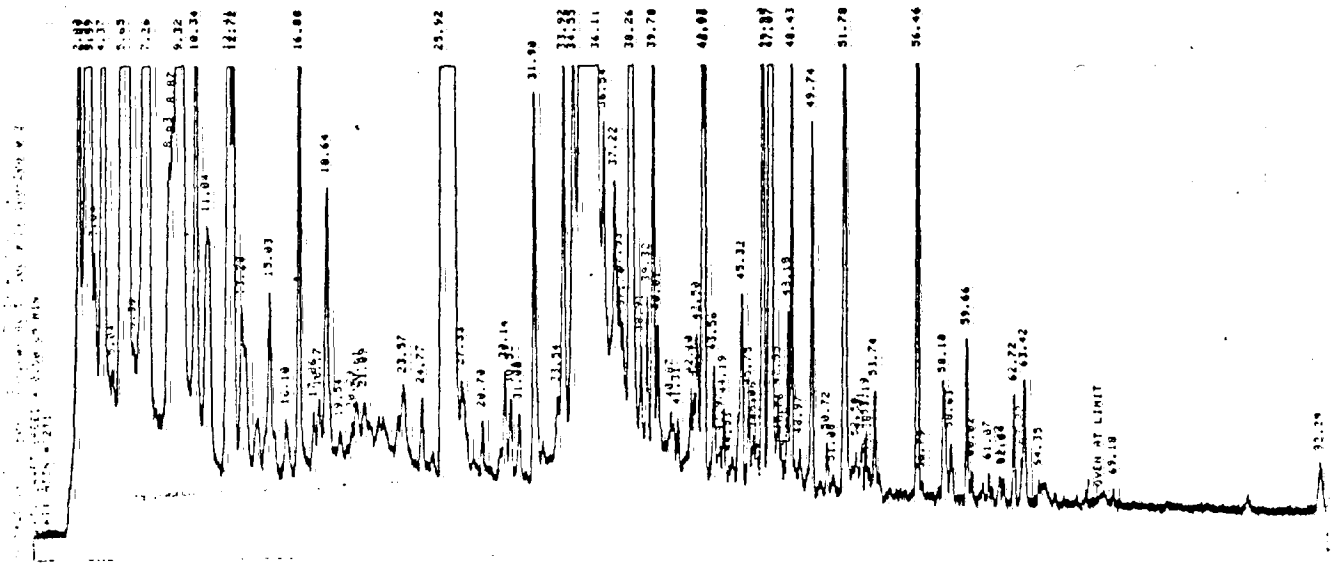
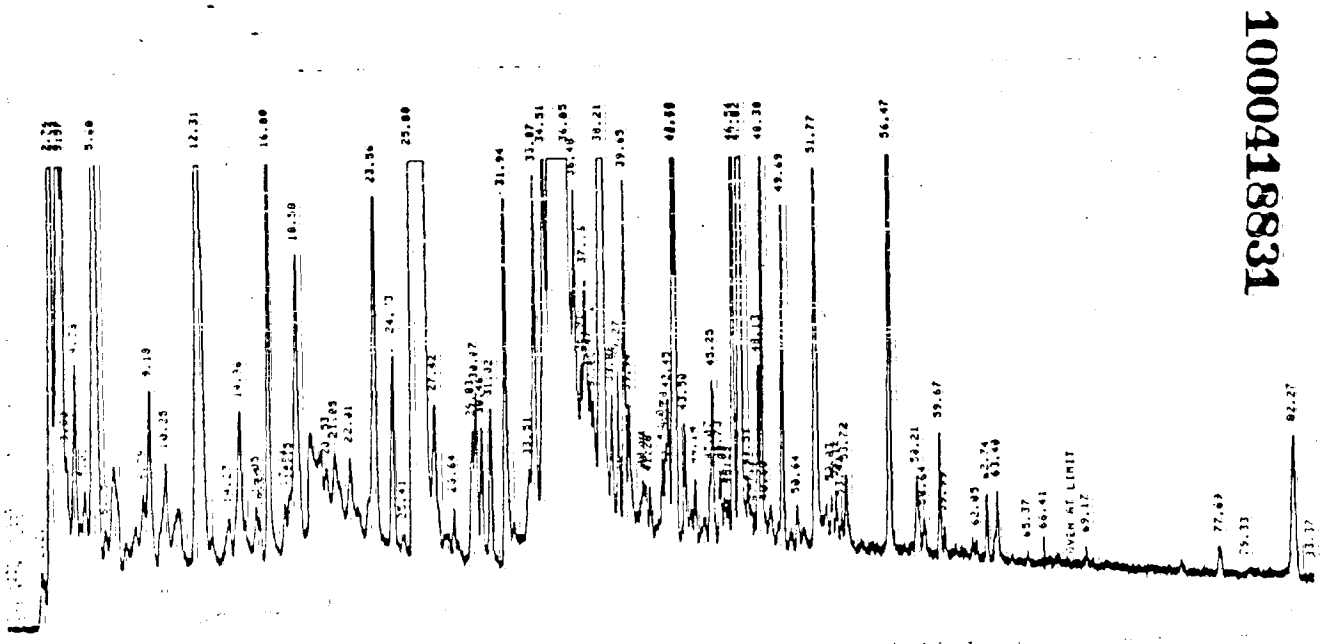
FIGURE 7

GAS CHROMATOGRAMS OF PLAYER'S CIGARETTES
IN PLAYER & B&H DELUXE UL BOXES



PLAYER'S CIGARETTES IN PLAYER BOX



PLAYER'S CIGARETTES IN B&H DELUXE UL BOX

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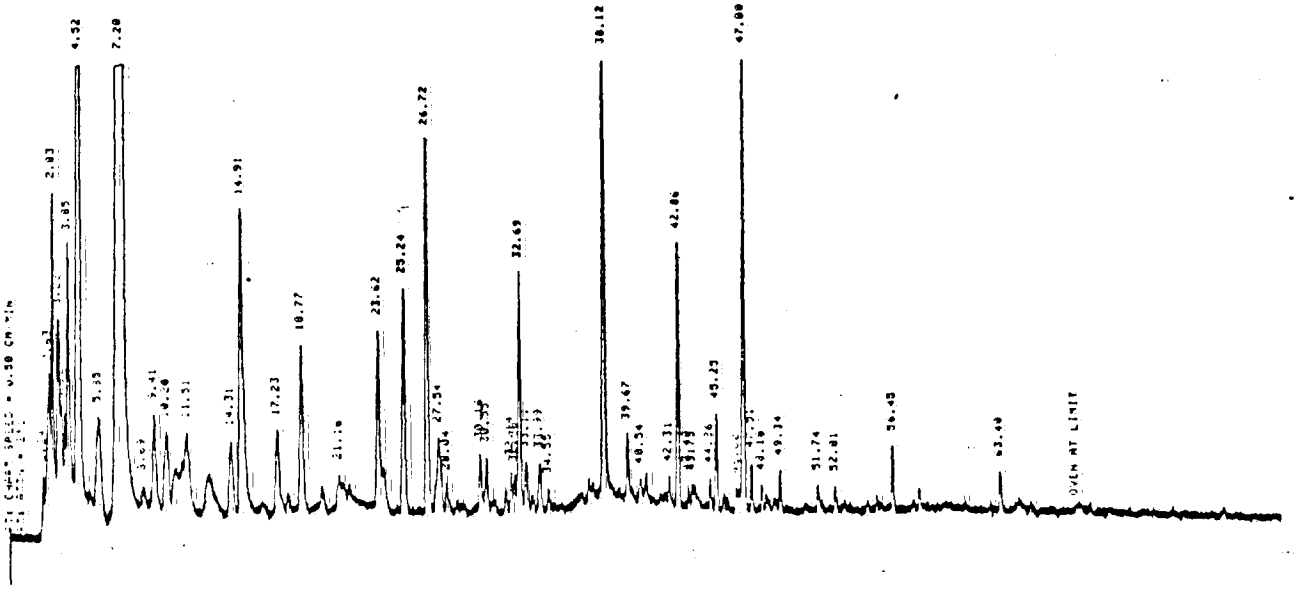
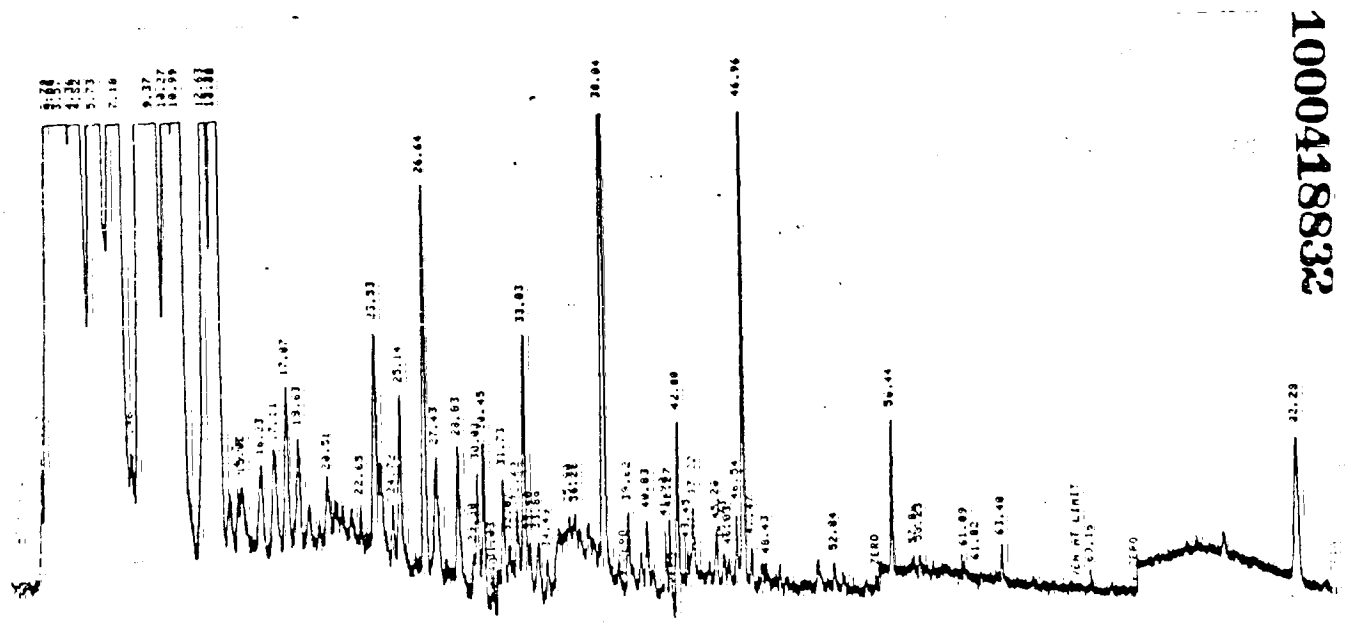
FIGURE 8

GAS CHROMATOGRAMS OF UNPRINTED AND PRINTED TAKO BOARDS



UNPRINTED



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cigarettes resembles the cigarettes taken from the box. The volatiles from the printed box and volatiles from the cigarettes are present. The Raffles box which was not in contact with cigarettes shows the early volatile components found in the packaged cigarettes. The unprinted box shows a much lower amount of these early volatiles. Therefore, these components are associated with the printing.

Because of the presence of volatiles material in the unprinted board, various constituents used to make this board were obtained and each constituent was analysed by P&T/GC.

The major contamination of the cigarettes is from the printing. Samples of all the inks and lacquers used in the printing of Players boxes were analyzed. The solvents in the inks and lacquers have retention times of the materials with early retention times in the packaged cigarettes.

The work that has been carried out on the Raffles and Players cigarettes emphasizes the need to evaluate the packaging material along with the new cigarette models. The transfer of a large amount of material to the cigarette from the packaging material does not imply a change in smoke flavor, but it certainly does raise a "red flag." The flavor response will depend on the compounds transferred to the cigarettes. Some compounds may be flavorless and can be tolerated at high amounts. Other compounds with high flavor response in very low amounts may not be detected by the P&T/GC procedure but may be very deleterious to the product.

8. Marlboro 85

Just prior to the problem of Players and Raffles cigarettes, an initial study was carried out to determine the possible loss of added volatiles during manufacturing. Marlboro filler was sampled

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at the exit of the aftercut cylinder and at the exit of the storage silo and cigarettes samples after packaging. These samples were analyzed by the P&T/GC analysis. The GC scans showed a reduction of some "peaks," especially the very volatile components between samples taken after the flavor cylinder and exiting the silo. However, a major difference was observed between the sample exiting the silo and the sample of filler taken from the packaged cigarettes. There were twenty additional "peaks" in the packaged cigarettes. Therefore, the packaging and/or adhesives are apparently contributing significantly to the headspace volatiles of packaged cigarettes.

C. Coumarin in Cigarettes

Due to the recent print media interest in coumarin in cigarettes, the P&T/GC scans made during the past several years were reexamined for the presence of coumarin based on its retention time. Various concentrations of coumarin were prepared in methanol and injected into Marlboro cigarettes. The injected cigarettes were analyzed by the P&T/GC procedure and the areas for coumarin peak were integrated for the various amounts of added coumarin. A standard curve was established. Additional competitors' cigarettes were analyzed if they had not been previously analyzed by the P&T/GC procedure. Table 4 lists the brands of cigarettes and amounts of coumarin detected. Also listed in this table are cigarette brands in which coumarin was not detected. Further details of this work have been presented in Special Report #83-132, "Coumarin in U.S. Cigarettes," authored by Allen Palmer.

D. Miscellaneous Applications

1. Barclay

The taste of U.S. and Swiss made Barclay cigarettes was found

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TABLE 4

COUMARIN IN CIGARETTES

BRAND	AMOUNT (ug/cigt.)	DATE
Now 85	75-100	3/82
100	20-30	3/82
Vantage 85	10-20	4/82
100	<10	4/82
85 Menthol	<10	4/82
100 Menthol	30-50	10/82
Ultra Light 85	20-30	9/82
100	20-30	9/82
85 Menthol	<10	7/82
100 Menthol	75-100	7/82
Bright 85 (Test Market)	75-100	7/82
85 (Nat'l Market)	20-30	12/82
100 (Test Market)	75-100	7/82
100 (Nat'l Market)	40-60	12/82
More Light 100	<10	5/82
More 120	<10	3/82
Doral II 85	<10	4/82
85 Menthol	10-20	4/82
Salem Ultra 100	<10	4/82
Winston Ultra Light 100	<10	3/82
Carlton 85 (Manuf. 3/80)	50-75	3/82
Lucky Strike Filter 85 SP	<10	12/6
Barclay 80	<10	1/83

CIGARETTES WITHOUT COUMARIN

BRAND	DATE
Camel 70	3/82
Light 80	3/82
85	3/82
Filters 85	3/82
Winston 85	4/82
Winston Light 85	3/82
Ultra Light 85	3/82
Salem 85	4/82
100	11/81
Lights 100	11/81
Carlton 85	3/82
100	3/82
Lucky Strike Filter 85 Box	12/6
Pall Mall 85	9/82
Estra Light 85	9/82
Tareyton 85	9/82
Lark 85	2/82
L & M Lights 85	9/82
Kent 85	11/81
100	5/82
III 85	11/81
Golden Lights 85	4/82
100	11/81
True 85	3/82
85 Menthol	4/82
Old Gold Filter 85	9/82
Triumph 85	11/81
100	11/81
Kool 80	11/81
Mild 85	4/82
Filter Kings 85	4/82
Lights 85	4/82
Super Lights 85	11/81
100	2/82
Ultra 85	11/81
100	2/82
Raleigh Lights 85	2/82

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to be quite different. The volatile scans were very different. Some of this difference could come from the packaging material.

2. Northwind

The loss of "Creme de Menthe" flavor peaks during accelerated aging was studied by P&T/GC.

3. Cabarrus Merit

P&T/GC volatile scans were made of a large number of Cabarrus-made Merit cigarettes in connection with a possible off-odor problem in the factory start-up.

4. Marlboro - Two Silo/Four Maker/4 Days

P&T/GC volatiles were examined on Marlboro filler sampled from M/C on four different days from two silos and four makers from each silo. There were some intensity differences in about 20 different peaks and this information was reported to Vivian Willis for possible correlation with subjective smoking results.

5. Marlboro - Overheated

Marlboro cigarettes which the filler may have been overheated were examined and the volatiles were slightly less than in the control, but probably not significantly less.

6. P&S Multipass Dryers

Volatiles of Marlboro, Merit and Burley fillers run thru the dryers at 150 and 170°F were compared. With Marlboro filler, there were 25 peaks with different intensities at the two temperatures, most of which were lower at the higher temperature. Merit had 20 and Burley 17 peaks whose intensity varied at the two temperatures, some being more intense and some less intense. Subjective smoking found differences in the Marlboro only.

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7. Dunhill Plastic Box

Dunhill boxes containing Merit regular and menthol cigarettes were subjected to accelerated aging and the boxes and cigarette fillers examined by P&T/GC. No volatiles from the boxes were detected in the fillers and no significant adsorption of filler volatiles was detected in the boxes. Additionally, four raw materials for making plastic boxes were examined for volatiles content.

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V. NICOTINE TRANSFER TO SMOKE[7]

A problem on the transfer of nicotine to smoke with Galaxy cigarettes in Brazil was presented to us several years ago. The problem was the Galaxy cigarette delivered nicotine to smoke more effectively than a similar cigarette, Advance, made by BAT. The cigarette data for these cigarettes are shown in Table 5.

TABLE 5

CIGARETTE ANALYSIS OF ADVANCE AND GALAXY CIGARETTES FROM BRAZIL

	Galaxy			Advance		
Tar, mg	11.9	12.1	10.3	11.0	12.2	9.4
Nicotine, mg	0.77	0.69	0.70	0.69	0.71	0.72
PUffs/Cigt.	7.7	7.3	7.5	7.7	7.3	7.7
Total Alkaloids, %	1.26	1.22	1.30	1.84	1.66	1.64
Filler Rod						
TPM, mg			27.3			25.6
Nicotine, Mg			1.08			1.17
Water, mg			6.0			5.0

In Brazil the public concern is more for nicotine than for tar delivery. Therefore, the Galaxy cigarette had a lower alkaloid content in the filler than BAT's Advance to deliver the same amount of nicotine. Mr. Wickham carried out experiments to determine the reason for the difference in nicotine delivery to smoke. He showed that the filters were not responsible for this difference by exchanging filters from the Advance and Galaxy cigarettes and also attaching Marlboro filters to both tobacco rods. Therefore, the difference in the nicotine transfer was due to the tobacco rod. Chemical analyses

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were carried out on the fillers from the two cigarettes and the results are shown in Table 6.

TABLE 6

FILLER AND SMOKE ANALYSIS OF GALAXY AND ADVANCE CIGARETTES FROM BRAZIL

Filler	Galaxy	Advance
K^+ (%)	3.7	4.0
Na^+ (%)	0.10	0.10
Mg^{++} (%)	0.42	0.46
Ca^{++} (%)	1.9	2.1
NO_3^- (%)	0.09	0.22
Cl^- (%)	0.22	0.25
$PO_4^=$ (%)	0.28	0.26
$SO_4^=$ (%)	0.95	0.98
Glycerin (%)	1.7	0.7
Tot. Alk. (%)	1.20	1.28
Tot. Red. Sugars (%)	10.3	9.8
Tot. Ash (%)	13.6	14.6
Stem Content (%)	27.4	26.6
Smoke		
Tar, mg/cigt.	9.4	7.8
Nicotine, mg/cigt.	0.74	0.58
Puff Count	7.4	7.6

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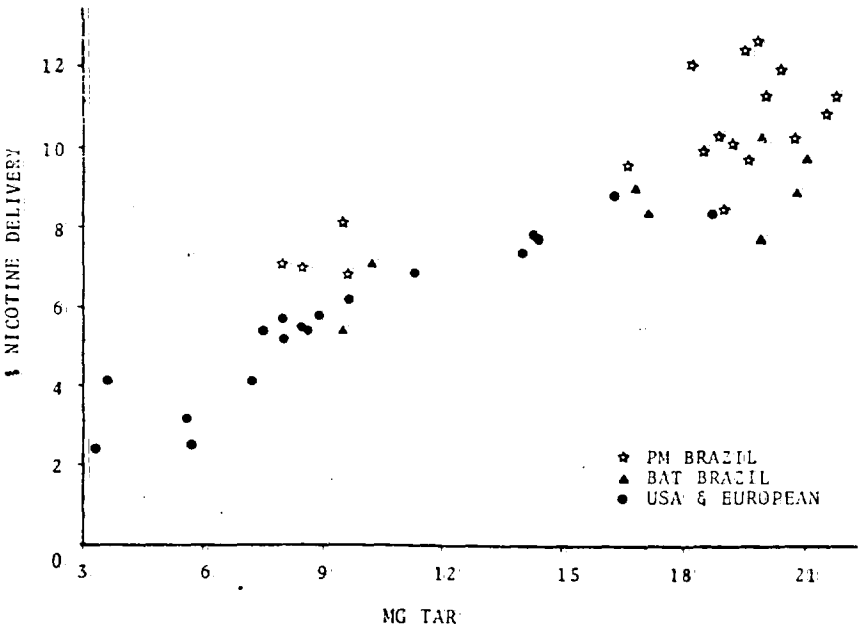
Nicotine delivery/total alkaloids ratio (or efficiency of nicotine delivery) was 0.57 for Galaxy and 0.41 for Advance for data from Table 5. More recent data, Table 6, confirms this. However, in this latest instance the Advance cigarette has maintained the ratio by reducing the total nicotine delivery by utilizing a tobacco having lower alkaloid content.

A plot was made of percent nicotine delivered to smoke versus tar. These values were calculated from the CI data for both US and European cigarettes and for PM and BAT cigarettes in Brazil. This plot is shown in Figure 9. The percent nicotine delivered was calculated from the nicotine delivered and the mg of alkaloids in the tobacco rod. The plot shows that the transfer of nicotine to smoke for the Advance cigarette is in line with US and European cigarettes while PM cigarettes in Brazil have a higher transfer of nicotine to smoke.

FIGURE 9

PERCENT NICOTINE DELIVERY VERSUS TAR



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A search of the literature and in-house reports revealed several chemical treatments of filler which would modify nicotine delivery. Cipriano [8] reported an increase in nicotine delivery when relatively high amounts of formic acid were added to filler. Thornton and Massy [9] reported a decrease in nicotine delivery by the addition of sugars to Burley tobacco. Mumpower and Kiefer [10] and Houck and Fondren [11] reported the reduction in nicotine delivery by the addition of glycerine to filters.

Cigarette models were prepared with various organic acids, sugars and glycerine to the filters. No significant difference in nicotine delivery was observed.

Salt casing study[12] showed that the addition of various salts such as nitrates, potassium acetate and citrate and magnesium sulfate reduced tar and nicotine. Analysis of nitrate-nitrogen in the two cigarettes from Brazil showed the Advance (0.22%) was higher than for the Galaxy (0.09%).

Dale Hill in the Leaf Department referred us to work carried out by Chaplin and Miner,[13] where the application of potassium was varied during the growing of tobacco. They showed that the higher the application of potassium to tobacco the lower the nicotine delivery to smoke. The results from their work are shown in Table 7.

A cooperative effort of this work with the Leaf Department on the application of potassium on tobaccos grown in Brazil is planned, and to determine their effect on nicotine delivery. Also, glycerine has been added to Galaxy filters to reduce nicotine delivery and the addition of nitrate to the level in Advance has been suggested.

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TABLE 7

EFFECT OF K APPLICATION

OF TOBACCO AND SMOKE

K_2O #/Acre	In Tobacco %		Smoke mg		Puff Count No.
	K	Total Alkaloids	Tar	Nicotine	
0	1.10	2.42	22.7	2.30	13.7
30	1.41	2.33	21.8	2.20	12.5
60	1.52	2.21	22.8	2.17	12.6
90	1.74	2.35	21.4	2.13	12.2
120	1.95	2.41	20.0	2.07	11.5
240	2.39	2.34	18.9	1.94	11.7

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VI. CASINGS AND FLAVORS

A. Vanilla Flavor Replacement

The work on the isolation and identification of the volatile components in vanilla to aid in the formulation of a substitute flavor was discussed in the last annual report. A Special Report #82-237, "Components of Vanilla Beans and Vanilla Extract," by R. W. Hale was issued.

B. Tonka Beans

Tonka bean extract has been used to flavor cigarettes primarily for its high coumarin content. Subjective evaluation of cigarettes flavored with Tonka bean extract and with pure coumarin has shown that coumarin lacks some of the desirable smoke flavor response of the Tonka bean extract. Thus, the purpose of this work was to identify the compounds in Tonka beans that impart its desirable flavor response.

Ground tonka bean samples were extracted with methylene chloride. Unexpected in the extract was an oil (probably a triglyceride) which represented about 25% of the weight of the original Tonka bean sample. This large amount of oil interferred in the identification of the minor components. To separate this oil and coumarin from the other volatile constituents, the extract was steam distilled in a Nickerson-Likens apparatus. However, a large amount of the oil and coumarin steam distilled. For further separation, the steam distillate was separated by silicic acid column chromatography into 12 fractions. These fractions were analyzed by GC/MS.

The residue after extracting the Tonka bean with methylene chloride was then extracted with water. The aqueous solution was extracted with methylene chloride to remove any residual methylene

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chloride soluble material. The extracted aqueous phase was acidified with HCl and heated to hydrolyze the water soluble constituents. This material was extracted with methylene chloride and the methylene chloride extract separated into 14 fractions by column chromatography. These fractions were analyzed by GC/MS.

A sample of Tonka beans was also extracted with 80% methanol and 20% water mixture to reduce the extraction of the oil. This extract was separated by column chromatography into 18 fractions and analyzed by GC/MS.

Compounds identified in this work are listed in Table 8. Most of the compounds identified in the Tonka beans have also been identified in tobacco.

TABLE 8

LIST OF COMPONENTS TENTATIVELY IDENTIFIED IN TONKA BEAN EXTRACTS

COUMARIN
DIHYDROCOUMARIN
4,5-DIMETHYL-4-HEXEN-3-ONE
4-HYDROXYBENZALDEHYDE
2-(2-BUTOXYETHOXY)-ETHANOL
2-TOLUALDEHYDE
OLEIC ACIDS
PHENETHYL ALCOHOL
HYDROXYACETOPHENONE
ETHYL OCTANOATE
3-METHOXYBENZALDEHYDE
DECANOL
PULEGONE
4-PENTEN-1-OL
FURYL METHYL KETONE
gamma-BUTYROLACTONE
2-METHYL-5-ETHYL FURAN
4-HYDROXY-2-PENTENOIC ACID LACTONE
BENZYL ACLOHOL
PHENOL
CRESOL
2-PHENETHYL ALCOHOL
2-PHENYL-2-PROPANOL
2-PHENETHYL FORMATE
TETRADECANOL
VANILLIN
bis-(1-METHYLPROPYL)-PHENOL
ISOAMYL BENZOATE
NONANOL
FURFURAL
GUAIACOL
4-ETHYLPHENOL
HYDROXYCOUMARIN
2,4-DIHYDROXYACETOPHENONE
2-METHOXY-5-VINYL PHENOL
8,9-DEHYDRO-4,5-DIHYDRO-THEASPIRONE
5-METHYL-2-FURFURAL
1,4,6-TRIMETHYL-2-PYRIDONE
EUGENOL
7,8-DEHYDRO-IONOL (?)
2,6-bis-(1,1-DIMETHYLETHYL)-4-METHYL PHENOL
SALICYL ALDEHYDE
METHYL FUROATE
ISOPROPYLPHENOL
4-(1,1-DIMETHYLETHYL)-PYROCATECHOL
1-BUTYL-1-BUTYRATE
PHENETHYL ACETATE
QUINOLINE
BENZTHIAZOLE
ACETOPHENONE
2-PHENOXYETHANOL
2,6,6-TRIMETHYL-CYCLOHEX-2-ENE-1,4-DIONE
2-METHYL-2-PENTENAL
CROTOLACTONE
BUTYRIC ACID
VALERIC ACIDS
2-TERPINEOL
DIBUTYL MALEATE
ISOPHORONE
2-BUTOXYETHANOL
2-ETHYL-1-HEXANOL
2-PENTYLFURAN
METHOXYPHENYL PROPANOL
HYDROXYMETHOXYPHENYL PROPENOL
BENZOFURAN
3,3,5,7-TETRAMETHYL-1-INDANONE
2-METHYL-4-HYDROXY-2-HEXENOIC ACID LACTONE
3,4,7-TRIMETHYL-1-INDANONE
FATTY ALCOHOLS
FATTY ACIDS
ESTERS

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Fractions isolated from the silicic acid column chromatography were given to the Flavor Creation Group and are presently being evaluated subjectively on cigarettes.

Details of this work can be found in Special Report #83-087, "Volatile Constituents of Tonka Bean Extracts," by R. W. Hale and J. R. Hearn.

C. Cocoa Shells[14]

The purpose of this work was to determine the role of cocoa shells in cigarette smoke flavor. The ground cocoa shells have an odor of chocolate. A sample of ground cocoa shells was extracted with methylene chloride. On concentration of the extract, a solid material resembling cocoa butter was obtained which represented approximately 20% of the weight of the cocoa shells. The extract had very little chocolate odor and was probably lost during concentration of the extract. The residue was further extracted with Freon-113 to concentrate the volatiles. The material extracted with the Freon was separated by packed column GC. The separated material was collected in melting point glass tubes and reanalyzed by capillary column GC/MS. The compounds identified are listed in Table 9.

TABLE 9

VOLATILE COMPOUNDS IDENTIFIED IN COCOA SHELL

METHYL STEARATE	2-METHOXY-6-METHYLPYRAZINE
ETHYL PALMITATE	A gamma LACTONE
METHYL PALMITATE	PHENETHYL ALCOHOL
NONADECANE	2-METHYL-3-HYDROXYPYRONE
TETRADECANE	MENTHOL
DOCOSANE	METHYL PHENYL ACETATE
TETRACOSANE	5-HYDROXYMETHYL-2-FURFURAL
HEPTADECANOL	TETRHYDROPYRAN
NONADECANOL	PHENYL ACETIC ACID
FARNESANE	5-PROPYL-PENT-2-ENE-5-OLIDE
TETRA METHYL NAPHTHALENE	STEARIC ACID
2-ACETYLPYRROLE	OCTANOL
2-FORMYLPYRROLE	DODECANOL
	CAFFEINE

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The amounts of the individual materials identified are estimated to be present in less than a ppm in the cocoa shells and would not contribute to cigarette flavor. At this time we do not know the role of the cocoa butter-like material which is present in considerable quantity. Work is planned to determine the flavor response of this material. A special report is being written on this work.

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VII. PLANS

A. Continue work on the isolation and identification of both volatile and nonvolatile components of tobacco. Explore better separation techniques for the nonvolatile fraction.

B. Evaluate the ultrasonically generated aerosol as a model system for subjectively evaluating nicotine and other flavor compounds for their odor and feeling factor sensations.

C. Utilize the purge and trap/GC technique for the analysis of volatile material.

1. Transfer of volatile compounds from packaging material to cigarette rod.
2. Analyze new competitor's cigarettes.
3. Monitor added flavors to new cigarette models during aging studies.

D. Explain the reason for the higher nicotine transfer to smoke for PM's Brazilian cigarettes.

E. Study the transfer of various flavor compounds to smoke.

F. Separate and identify compounds in selected flavors used in manufacturing to aid in the formulation of substitute flavors.

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VIII. ACKNOWLEDGMENTS

We wish to acknowledge a number of other groups in the R&D Center who have assisted us in our program during the past year. These include the other groups in the Flavor Development Division who have subjectively evaluated fractions isolated from tobacco and members of the Analytical Research Division who have assisted in the identification of compunds in fractions isolated from tobacco.

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Source: https://www.industrydocuments.ucsf.edu/docs/fmnl0000

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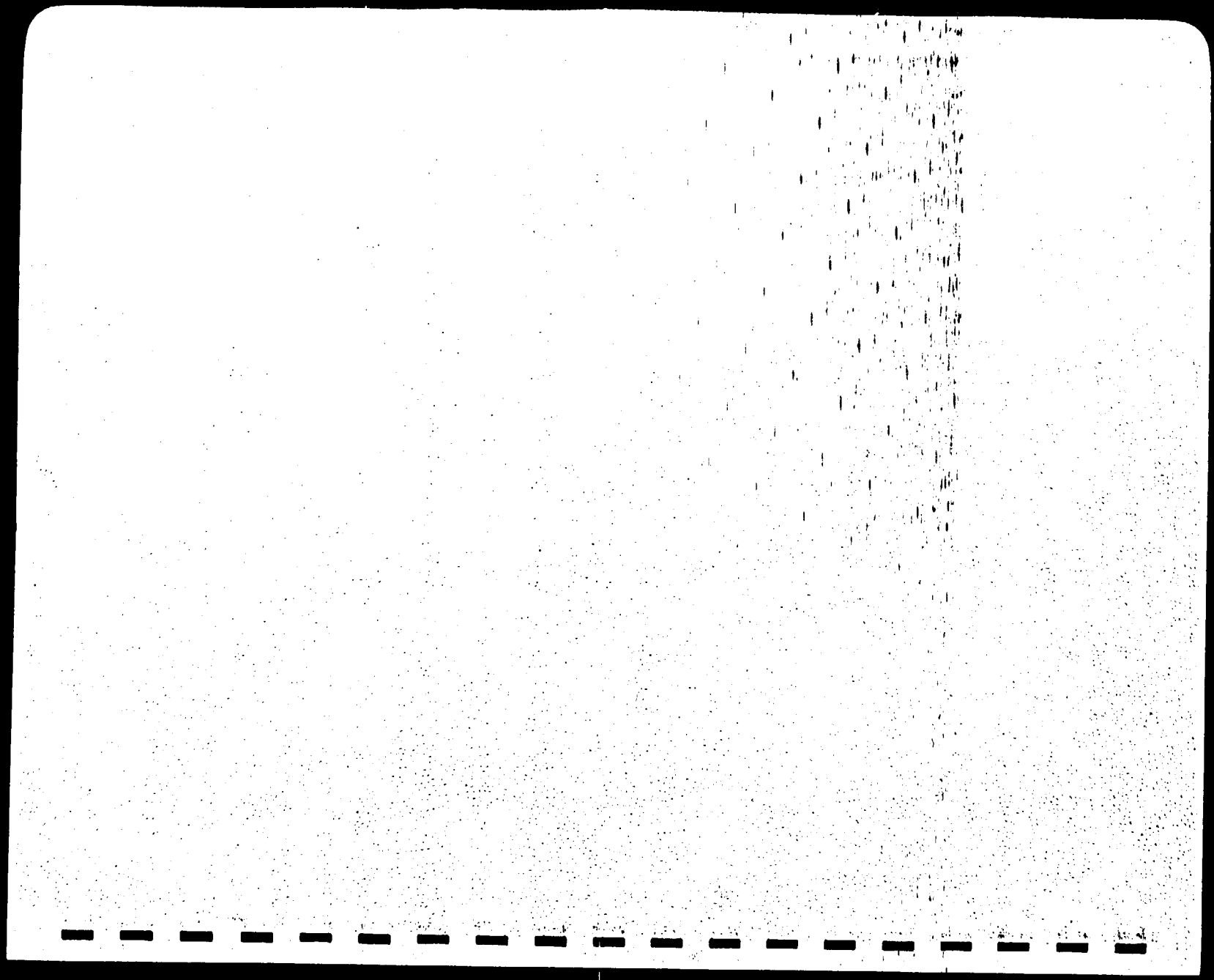

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